

Full Length Research Paper

Early prediction of cardiovascular diseases, a lifestyle disorder, in software people in south India using high sensitive c reactive protein (hsCRP)

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The study was aimed to find out the incidence of cardiovascular diseases in young software professionals. Coronary artery block or arteriosclerosis was considered to be a form of inflammation. High sensitive C - reactive protein (hsCRP) is considered to be one of the sensitive markers of systemic inflammation, which has also been associated with an increased risk of cardiovascular disease. Software professional have a sedate working style, more of mind working than physical work. This is same for both males as well as females and is further aggravated due to some unwanted habits like smoking and alcoholism. The population was categorized into 3 depending on the age group of less than 30 years, as in case of males and females and below 45 years as in case of males. The levels of cholesterol, triglycerides, high density lipids (HDL), low density lipids (LDL) and C - reactive protein (CRP) along with hsCRP values were evaluated. Data regarding smoking and alcoholism were also documented. The present study shows that in males below 30 years, hsCRP levels were elevated in people having elevated levels of LDL. Simultaneously in women below 30 years, who had high hsCRP levels also had increased LDL levels, but this relation of hsCRP and LDL is more marked in case of males than females. CRP levels were also found to be elevated in those people having elevated levels of hsCRP. These high levels of cholesterol and LDL are associated with the sedate working style of software professionals.

Key words: Software professionals, arteriosclerosis, high density lipids, low density lipids, high sensitive C - reactive protein (hsCRP), cholesterol, triglycerides.

INTRODUCTION

C - reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation (an acute-phase protein). Its physiological role is to bind to CRP was originally discovered by Tillet and Francis (1930) as a substance in the serum of patients with acute inflammation that reacted with the C polysaccharide of *Pneumococcus*. Initially it was thought that CRP might be

a pathogenic secretion as it was elevated in people with a variety of illnesses including cancer, however discovery of hepatic synthesis demonstrated that it is a native protein.

CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body (Patel et al., 2001). This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes. CRP rises up to 50,000 - fold in acute inflammation, such as infection. It rises above normal limits within 6 h, and peaks at 48 h (Pearson et al., 2003).

A high-sensitivity CRP (hs-CRP) test measures low levels

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of CRP using nephelometry. Normal concentration in healthy human serum is usually lower than 10 mg/L, slightly increasing with ageing. Higher levels are found in late pregnant women; mild inflammation and viral infections (10 to 40 mg/L), active inflammation, bacterial infection (40 to 200 mg/L), severe bacterial infections and burns (> 200 mg/L) (Ridker 2003). A growing number of studies have examined whether hs-CRP can predict recurrent cardiovascular disease, stroke and death in different settings (Hopkins and Williams, 1981).

High levels of hs-CRP consistently predict recurrent coronary events in patients with unstable angina and acute myocardial infarction (heart attack). If hs-CRP level is lower than 1.0 mg/L, a person has a low risk of developing cardiovascular disease. If hs-CRP is between 1.0 and 3.0 mg/L, a person has an average risk. If hs-CRP is higher than 3.0 mg/L, a person is at high risk.

MATERIALS AND METHODS

The study was carried out on both males and females categorized under the age groups of below 30 years and above 45 years. A total group of 300 people were analyzed of which 100 were men < 30 years, 100 women < 30 years and 100 men between 30 and 45 years.

Data collection

The data of selected individuals were collected, the data's collected include; ages, sex, smokers and alcoholic

Sample collection and preparation

The blood samples were drawn using 5 ml disposable syringes from the vein. The blood samples were collected in test tubes coated with clot activator and gel, this enhances the blood clotting process and during centrifugation the gel separates the cellular component of the blood from the serum.

Testing

All the collected samples were subjected to various biochemical testing. The various tests performed included cholesterol, triglycerides, high density lipids (HDL), Low density lipids (LDL), CRP, hsCRP. All the biochemical testing was done using automated clinical chemistry analyzer – Miura. All the reagents were ready to use reagents and, the samples were added to the reagents in the respective proportion as per the instruction of the manufacturer and incubated and the optical density (O.D) was measured against the standard. The CRP and hs CRP tests were done by immunoturbidometric method using Miura clinical chemistry analyzer.

The samples were mixed with the hsCRP reagent and the turbidity developed was measured using automated clinical chemistry analyzer Miura.

Analysis of serum CRP

The serum sample 5 µl was mixed with the CRP kit (Futura

systems) 200 µl. The absorbance was measured at 546 nm chemistry analyzer MIURA (ISE.srl). The absorbance is plotted against a calibration curve to obtain the sample value. The results obtained are tabulated.

$$\text{CRP concentration (mg/L)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times \text{Standard concentration}$$

Analysis of Serum hsCRP

The serum sample 5 µl was mixed with the hsCRP kit (Futura systems) 200 µl. The absorbance was measured at 546 nm chemistry analyzer MIURA (ISE.srl). The absorbance is plotted against a calibration curve to obtain the sample value. The results obtained are tabulated.

$$\text{hsCRP concentration (mg/L)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times \text{Standard concentration}$$

Analysis of serum cholesterol

Total cholesterol (cholesterol oxidase peroxidase method)

10 µl of the sample was mixed with 1000 µl of working reagent (Cholesterol esterase > 180 U / L, Cholesterol oxidase > 200 U / L, peroxidase > 1000 U / L). It was mixed well and incubated for 5 min at 37°C. Then the absorbance was measured at 505 nm. Cholesterol concentration is calculated as follows:

$$\text{Cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times 200$$

Where '200' is the standard concentration.

Analysis of HDL-cholesterol (Immuno inhibition method)

5 µl of the sample was mixed with 450 µl of reagent 1 (Good's buffer pH 7.0 30 mM / L, 4 -aminoantipyrine – 0.9 mM/L, POD 2.4 IU / ml). It was mixed well and incubated for 5 min at 37°C. Then 150 µl of reagent 2 (Good's buffer pH 7.0, 30 mM / L, cholesterol esterase 4 IU / ml, Cholesterol oxidase 20 IU / ml) was added to the previous mixture. Again it was mixed well and incubated for 5 min at 37°C and the absorbance was measured at 600 nm.

$$\text{HDL concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of calibrator}} \times \text{calibrator concentration}$$

Where '60' is the calibrator concentration.

Analysis of LDL-cholesterol (Enzyme Selective protection method)

5 µl of the sample was mixed with 450 µl of reagent 1 (Good's buffer pH 6.8 25 mM / L, cholesterol esterase 5 IU / ml, cholesterol oxidase 5 IU / ml, catalase 1000 IU / ml). It was mixed well and incubated at 37°C for 5 min. To that 150 µl of reagent 2 (Good's

buffer pH 7.1, 30 mM / L, 4 -aminoantipyrine 3.4 mM / L, peroxidase 20 IU / ml and sodium azide 0.1%) was added. It was again mixed well and incubated at 37°C for 5 min. Then the absorbance was read at 600 nm.

$$\text{LDL concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of calibrator}} \times \text{calibrator concentration}$$

where '80' is the calibrator concentration.

Analysis of triglycerides (GPO-PAP method)

10 µl of sample was mixed with 100 µl of reagent (p-chlorophenol 5.3 mM / L, potassium ferrocyanate 10 µ mol / L, magnesium salt 17 mM / L, 4-amino antipyrine 0.9 mM / L, lipoprotein lipase ≥ 1800 U / L, Glycerol kinase ≥ 450 U / L, Glycerol-3 phosphate oxidase ≥ 3500 U/L, peroxidase ≥ 450 U/L). It was mixed well and incubated at 37°C for 5 min. Then the absorbance was read at 505 nm.

$$\text{Triglycerides concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard concentration}$$

where '200' is the standard concentration.

Analysis of very low density lipids (VLDL)

The serum triglyceride values were divided by 5, or in other words the very low density lipids concentration in serum will be 1/5 th of the triglyceride concentration in the serum.

$$\text{VLDL concentration (mg/dl)} = \frac{\text{Triglyceride Conc. in sample}}{5}$$

Screening for alcoholism

All the 300 software professionals who participated in the screening program were asked if they have a habit of consuming alcohol; the reply obtained were, occasional drinkers and regular drinkers, but in the present study, the people were classified into two categories either alcohol consuming or non consuming, even the occasional drinkers were considered to be alcoholics and the results of which are tabulated.

Screening for smokers

All the 300 software professionals who participated in the screening program were asked, if they had a habit of smoking of any kind. The replies obtained was, occasional smokers and regular smokers; however in the study all the people who smoked were classified under smokers and who did not smoke even one were classified under nonsmokers, even the occasional smokers were considered to be smokers .The results of which are tabulated.

RESULTS

100 females below 30 years

Cholesterol

From the results obtained, out of the 100 samples, 66 participants were found to be normal, having a cholesterol level of < 200 mg/dl. 19 participants were found to be under suspected category, having a cholesterol level between 200 to 250 mg/dl and 15 participants were found to fall under the category of pathological that is having cholesterol levels higher than > 250 mg/dl. The results are tabulated in Table 1.

Triglycerides

From the results obtained, out of the 100 samples, 99 participants were found to be normal, having a triglycerides level between 50 to 165 mg/dl. 1 participant was found to be under the category of pathological, that is having triglycerides levels higher than >165 mg/dl. The results are tabulated in table 1.

High density lipoprotein (HDL)

From the results obtained, out of the 100 samples, 27 participants were found to be having Good control, having a HDL cholesterol level of > 50 mg/dl. 27 participants were found to be under Normal category, having a HDL cholesterol level between 35 to 50 mg/dl and 46 participants were found to fall under the category of high risk that is having HDL cholesterol levels less than < 35 mg/dl, (Table 1).

Low density lipoprotein (LDL)

From the results obtained, out of the 100 samples, 61 participants were found to be having good control, having a LDL cholesterol level of < 130 mg/dl. 13 participants were found to be under medium risk category, having a LDL cholesterol level between 130 to 159 mg/dl and 26 participants were found to fall under the category of high risk, that is, having LDL cholesterol levels less than > 160 mg/dl, as shown in Table 1.

Very low density lipoprotein (VLDL)

From the results obtained, out of the 100 samples, 97 participants were found to be having normal levels that is having a VLDL cholesterol level of < 30 mg/dl.

Table 1. Screening of adults of age below 30 years of females (Conroy et al., 2003).

S/N	Level	Concentration	Category	Result
1	hsCRP	< 1 mg/L	Good	46
		1 - 3 mg/L	Normal	41
		> 3 mg/L	High	13
2	HDL	> 50 mg/dl	Good	27
		35-50 mg/dl	Normal	27
		< 35 mg/dl	High	46
3	LDL	< 130 mg/dL	Good	61
		130 - 159 mg/dl	Medium risk	13
		> 160 mg/dl	High risk	26
4	CHL	> 250	Pathological	15
		200 - 250	Suspected	19
		< 200	Normal	66
5	VLDL	> 30	High	3
		< 30	Normal	97
6	TGL	> 165	High	1
		50 - 165	Normal	99
7	CRP	< 6 mg/L	Normal	64
		> 6 mg/L	Abnormal	36

Three participants were found to be under High risk category, having a VLDL cholesterol level between > 30 mg/dl.

C-Reactive protein (CRP)

From the results obtained, out of the 100 samples, 64 participants were found to be having normal, CRP level of < 6 mg/L. 36 participants were found to be under Abnormal category, having a CRP level more than > 6 mg/L.

High sensitive C - reactive protein (hsCRP)

From the results obtained, out of the 100 samples, 46 participants were found to be normal levels, having a hsCRP level of < 1 mg/L. 41 participants were found to be under Medium risk category, having a hsCRP level between 1 to 3 mg/L. and 13 participants were found to fall under the category of High risk, that is having hsCRP levels less than > 3 mg/L.

Alcoholic

Out of the 100 participants, no one admitted of having habit of consuming alcohol, not even occasionally.

Smoking

Out of the 100 participants, no one admitted of having habit of smoking, not even occasionally.

100 males below 30 years

Cholesterol

From the results obtained, out of the 100 samples, 58 participants were found to be normal, having a cholesterol level of < 200 mg/dl. 12 participants were found to be under suspected category, having a cholesterol level between 200 to 250 mg/dl and 24 participants were found to fall under the category of pathological that is having cholesterol levels higher than > 250 mg/dl (NCEP, 2002). The results are tabulated in

Table 2. Screening of adults of age below 30 years of males (Conroy et al., 2003).

S/N	Level	Concentration	Category	Result
1	hsCRP	< 1 mg/L	Good	49
		1 - 3 mg/L	Normal	38
		> 3 mg/L	High	13
2	HDL	> 50 mg/dl	Good	27
		35 - 50 mg/dl	Normal	41
		< 35 mg/dl	High	32
3	LDL	< 130 mg/dl	Good	51
		130 - 159 mg/dl	Medium risk	20
		> 160 mg/dl	High risk	29
4	CHL	> 250	Pathological	24
		200 - 250	Suspected	12
		< 200	Normal	58
5	VLDL	> 30	High	22
		< 30	Normal	78
6	TGL	> 165	High	12
		50 - 165	Normal	88
7	CRP	< 6 mg/L	Normal	53
		>6 mg/L	Abnormal	47

Table 2.

Triglycerides

From the results obtained, out of the 100 samples, 88 participants were found to be normal, having a triglycerides level between 50 to 165 mg/dl. 12 participants were found to be under the category of pathological that is having triglycerides levels higher than > 165 mg/dl, as shown in Table 2.

High density lipoprotein (HDL)

From the results obtained, out of the 100 samples, 27 participants were found to be having Good control, having a HDL cholesterol level of > 50 mg/dl. 41 participants were found to be under normal category, having a HDL cholesterol level between 35 to 50 mg/dl and 32 participants were found to fall under the category of high risk that is having HDL cholesterol levels less than < 35 mg/dl.

Low density lipoprotein (LDL)

From the results obtained, out of the 100 samples, 51 participants were found to be having good control, having a LDL cholesterol level of < 130 mg/dl. 20 participants were found to be under medium risk category, having a LDL cholesterol level between 130 to 159 mg/dl and 29 participants were found to fall under the category of High risk, that is having LDL cholesterol levels less than > 160 mg/dl.

Very low density lipoprotein (VLDL)

From the results obtained, out of the 100 samples, 78 participants were found to be having Normal levels that is having a VLDL cholesterol level of < 30 mg/dl. 22 participants were found to be under high risk category, having a VLDL Cholesterol level between > 30 mg/dl.

C-reactive protein (CRP)

From the results obtained, out of the 100 samples, 53 participants were found to be having normal, CRP level of

< 6 mg/L. 47 participants were found to be under abnormal category, having a CRP level more than > 6 mg/L.

High sensitive C-reactive protein (hsCRP)

From the results obtained, out of the 100 samples, 49 participants were found to be normal levels, having a hsCRP level of < 1 mg/L. 38 participants were found to be under Medium risk category, having a hsCRP level between 1 to 3 mg/L and 13 participants were found to fall under the category of High risk, that is having hsCRP levels less than > 3 mg/L.

Alcoholic

Out of the 100 participants, 57 participants admitted of having habit of consuming alcohol, and 43 participants never consumed alcohol not even occasionally.

Smoking

Out of the 100 participants, 36 participants admitted of having habit of smoking and 64 participants never smoked, not even occasionally.

100 males between 30 to 45 years

Cholesterol

From the results obtained, out of the 100 samples, 43 participants were found to be normal, having a cholesterol level of < 200 mg/dl. 25 participants were found to be under suspected category, having a cholesterol level between 200 to 250 mg/dl and 32 participants were found to fall under the category of pathological, that is having cholesterol levels higher than > 250 mg/dl.

Triglycerides

From the results obtained, out of the 100 samples, 85 participants were found to be normal, having a triglycerides level between 50 to 165 mg/dl. 15 participants were found to be under the category of pathological that is having triglycerides levels higher than > 165 mg/dl. The results are tabulated in table 3.

High density lipoprotein (HDL)

From the results obtained, out of the 100 samples, 33

participants were found to be having Good control, having a HDL cholesterol level of > 50 mg/dl. 55 participants were found to be under Normal category, having a HDL cholesterol level between 35 to 50 mg/dl and 12 participants were found to fall under the category of High risk that is having HDL Cholesterol levels less than < 35 mg/dl. The results are tabulated in Table 3.

Low density lipoprotein (LDL)

From the results obtained, out of the 100 samples, 35 participants were found to be having Good control, having a LDL cholesterol level of < 130 mg/dl. 26 participants were found to be under Medium risk category, having a LDL cholesterol level between 130 to 159 mg/dl and 39 participants were found to fall under the category of High risk that is having LDL cholesterol levels less than > 160 mg/dl.

Very low density lipoprotein (VLDL)

From the results obtained, out of the 100 samples, 72 participants were found to be having normal levels that is having a VLDL cholesterol level of < 30 mg/dl. 28 participants were found to be under High risk category, having a VLDL cholesterol level between > 30 mg/dl.

C-reactive protein (CRP)

From the results obtained, out of the 100 samples, 53 participants were found to be having normal CRP level of < 6 mg/L. 47 participants were found to be under abnormal category, having a CRP level more than > 6 mg/L.

High sensitive C-reactive protein (hsCRP)

From the results obtained, out of the 100 samples, 12 participants were found to be normal levels, having a hsCRP level of < 1 mg/L. 70 participants were found to be under Medium risk category, having a hsCRP level between 1 to 3 mg/L. and 18 participants were found to fall under the category of High risk, that is having hsCRP levels less than > 3 mg/L, as shown in Table 3.

Alcoholic

Out of the 100 participants, 55 participants admitted of having habit of consuming alcohol, and 45 participants never consumed alcohol not even occasionally.

Table 3. Screening of adults of age below 45 years of males (Conroy et al., 2003).

S / N	Levels	Concentration	Category	Result
1	hsCRP	< 1 mg/L	Good	12
		1 - 3 mg/L	Normal	70
		> 3 mg/L	High	18
2	HDL	>50 mg/dl	Good	33
		35 - 50 mg/dl	Normal	55
		< 35 mg/dl	High	12
3	LDL	< 130 mg/dl	Good	35
		130 - 159 mg/dl	Medium risk	26
		> 160 mg/dl	High risk	39
4	CHL	> 250	Pathological	32
		200 - 250	Suspected	25
		< 200	Normal	43
5	VLDL	> 30	High	28
		< 30	Normal	72
6	TGL	> 165	High	15
		50 - 165	Normal	85
7	CRP	< 6 mg/L	Normal	53
		> 6 mg/L	Abnormal	47

Smoking

Out of the 100 participants, 32 participants admitted of having habit of smoking and 68 participants never smoked, not even occasionally.

Conclusion

Inflammation is a major mechanism in the process of atherogenesis and in triggering of clinical cardiovascular events (Assmann et al., 2002). Some experts propose that CRP, a nonspecific marker of inflammation, is a new tool that improves cardiovascular risk estimation and that it should be used as a routine clinical risk assessment test. It is possible that CRP might add more predictive value by further stratifying or reclassifying risk among certain subgroups (Pepe et al., 2004).

The present study shows that in males below 30 years, the hsCRP levels were elevated in those people having elevated levels of LDL. Simultaneously in the women below 30 years, the samples which had high hsCRP levels also showed increased LDL levels, but this relation of

hsCRP and LDL is more marked in case of males than females.

Similarly, the hsCRP levels were found to be elevated in those people having smoking and alcoholic habits. This is a direct indication that these people have more chances of cardiovascular diseases than the non smokers and alcoholics. The CRP levels were also found to be elevated in those people having elevated levels of hsCRP. These high levels of cholesterol and LDL (bad cholesterol) are associated with the sedate working style of software professionals.

The levels of lipids were high in the men below the age group of 45 years, and considerably the hsCRP levels were also elevated. Out of the tested people belonging to age below 30 males, 20% of them had average risk and 20% of them have high risk of developing cardiac related problems in the future.

Out of the tested people belonging to age below 30 females, 50% of them had average risk of developing cardiac related problems in the future. Out of the tested people belonging to age below 45 males, 39% of them had average risk of developing cardiac related problems in the future.

From the previous screening study on software professionals, it is clear that there is a high risk of cardiovascular diseases associated with software professionals and hsCRP in correlation with the traditional cardiac markers adds value in predicting the future chances of cardiac diseases.

Abbreviations: **LDL**, Low density lipids; **HDL**, high density lipids; **hsCRP**, high sensitive C - reactive protein. phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via c1q. CRP is synthesized by the liver in response to factors released by fat cells (adipocytes). It is a member of the pentraxin family of protein. It is not related to C-peptide or protein C.

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